Underdiagnosis of cervical intraepithelial neoplasia by colposcopy and its association with thin high-grade squamous intraepithelial lesions

MINGZHU LI[1*], XIAOBO ZHANG[2*], QISONG ZHANG[2], YUN ZHAO[1], CHAO ZHAO[1], JINGRAN LI[1], HONG TAO[3], DANHUA SHEN[2] and LIHUI WEI[1]

Departments of [1]Obstetrics and Gynecology, and [2]Pathology, Peking University People's Hospital, Beijing 100044; [3]Hoomya Ltd., Changsha, Hunan 410017, P.R China

Received November 27, 2022; Accepted April 24, 2023

DOI: 10.3892/ol.2023.13873

Abstract. The relationship between the thickness of the epithelium and the colposcopic diagnosis is controversial. The present study was conducted to determine whether colposcopic underdiagnosis of cervical intraepithelial neoplasia (CIN) is associated with thin high-grade squamous intraepithelial lesions (HSILs) of the cervix. A total of 136 cases of HSIL verified by pathological biopsy at Peking University People's Hospital between June and October 2021 were retrospectively analyzed; 79 cases were CIN2 and 57 cases were CIN3. The number and thickness of epithelial layers were analyzed using colposcopic impressions. In the low-grade colposcopic impression group, the number of epithelial layers (12.8±4.2 vs. 17.8±4.2) and epithelial thickness (105.2±41.9 μm vs. 150.3±50.0 μm) of CIN2 lesions were significantly lower compared with the high-grade colposcopic impression group; however, the differences for CIN3 were not statistically significant. CIN2 lesions had significantly fewer (12.8±4.2 vs. 17.2±5.4) and thinner (105.2±41.9 μm vs. 140.4±48.6 μm) epithelial layers than CIN3 lesions in the low-grade colposcopic impression groups. In the high-grade colposcopic impression group, however, there were no significant differences in the number or thickness of epithelial layers between CIN2 and CIN3. In 12 cases of thin HSILs, 91.6% of the colposcopic impressions were low-grade. Thin HSILs are likely associated with underdiagnosed colposcopic findings, particularly for CIN2. Thin HSILs usually present with small to minute lesions and lack the typical colposcopic appearance of classic HSIL, which may help to explain why thin HSILs are easily underestimated under colposcopy.

Correspondence to: Dr Lihui Wei, Department of Obstetrics and Gynecology, Peking University People's Hospital, 11 Xizhimen South Street, Beijing 100044, P.R. China
E-mail: weilh@bjmu.edu.cn

Dr Danhua Shen, Department of Pathology, Peking University People's Hospital, 11 Xizhimen South Street, Beijing 100044, P.R. China
E-mail: danhuashen@126.com

*Contributed equally

Key words: high squamous intraepithelial lesions, thin, layer, thickness, colposcopic impression, underdiagnosis

Introduction

In 2020, the World Health Organization (WHO) updated its classification of female reproductive organ tumors from the original three-level classification of cervical intraepithelial neoplasia (CIN1, CIN2 and CIN3) to a two-level classification: Low-grade squamous intraepithelial lesions (LSIL/CIN1) and high-grade squamous intraepithelial lesions (HSILs) (1). HSILs may be subdivided into HSIL (CIN2) and HSIL (CIN3) (1), particularly in young women (aged <30 years), because there is evidence that the former show significantly higher regression rates (2). Exophytic LSILs are caused by low-risk human papillomavirus (LR-HPV) types, whereas 80-90% of flat LSILs are attributable to high-risk HPV (HR-HPV) types (1). The colposcopy findings for cases of LSILs usually involve thin or translucent whitening, often accompanied by geographic, condylomatous, raised, or papillary changes. These changes may or may not be accompanied by fine mosaic and/or punctation patterns (3). HSIL is recognized as a true precancer with a higher risk of progression than LSIL; it is usually caused by persistent HR-HPV infection and colposcopies frequently show typical dense acetowhite changes, with or without vascular changes (coarse mosaic and/or punctation) (3). However, these changes might not be observable for populations with borderline cytologic abnormalities accompanied by small, early lesions (4).

The relationship between the thickness of the epithelium and the colposcopic diagnosis is controversial. It has been previously reported that the inability to visualize certain HSILs is associated with a thinner epithelium (5). However, it has also been suggested that false-negative colposcopy in the presence of high-grade CIN is likely due to the failure to detect minor or hidden lesions in the cervical canal rather than the presence of a 'thin' CIN (6). In the present study, it was investigated whether a thin HSIL is associated with a less abnormal (i.e., thin acetowhite change) or low-grade colposcopic impression.

Materials and methods

Study subjects. A total of 136 cases of HSIL verified by pathological biopsy at Peking University People's Hospital between June and October 2021 were analyzed retrospectively. The study was approved by the Ethics Committee of Peking University People's Hospital (IRB number: 2020PHB298-01, date of IRB approval: 30/10/2020).

Pathological analysis. Pathology slides were scanned and digitized using a PRECICE 500B digital scanner (Beijing Una Technology Co., Ltd.). The thickness of the squamous epithelium was determined by measuring the distance between its surface and basement membrane. Average epithelial thickness was determined as follows: Thickness=(thickness of the thickest part + thickness of the thinnest part)/2 (6). A demonstration of the measurement of average epithelial thickness is presented in Fig. 1. A classic HSIL has a thickness of >10 cell layers, whereas a thin HSIL is described by the WHO as a cervix HSIL variant with a thickness of <9 cells (7). p16 and Ki-67 immunohistochemical staining was performed when the CIN grade could not be determined using morphology. All pathological specimens were fixed using 4% neutral formaldehyde at room temperature for 120 min, before the sample was conventionally dehydrated (graded alcohol series) and soaked, embedded in paraffin and sectioned (4 μm) for hematoxylin and eosin (HE) staining at room temperature for 10 min. All immunohistochemical staining was performed according to the manufacturers' protocols. Formalin-fixed paraffin-embedded blocks were sectioned at 4 μm each and incubated with antibodies (37˚C; 20-30 min). Immunohistochemistry was performed with the Ventana Benchmark XT-automatic staining machine (Roche Tissue Diagnostics). For $p16^{INK4a}$ detection, the CINtec Histology Kit (cat. no. 705-4713; clone E6H4; 1:100; Roche Diagnostics GmbH) was used following the manufacturer's protocol. Ki-67 immunohistochemistry was performed using rat anti-human monoclonal antibody (cat. no. EP5; clone UMAB107; 1:200; Origene Technologies, Inc.). Secondary antibody (HRP; ready-to-use; cat. no. ZLI-9013; Origene Technologies, Inc.) incubation was for 20-30 min at 37˚C.

The number and thickness of epithelial layers were measured by pathologists who were blinded to the HPV type, colposcopic diagnosis or prior histologic diagnosis of patients.

Colposcopy evaluation. Colposcopic impressions of these cases were retrospectively analyzed. Colposcopic impressions were performed according to the 2017 American Society for Colposcopy and Cervical Pathology (ASSCP) standard (8). Absence of an acetowhite abnormality was defined as 'normal'. The presence of thin acetowhite lesions, either on or off the squamocolumnar junction (SCJ), indicated possible metaplasia or LSIL and was defined as a 'low-grade impression group'. HSIL was indicated by the presence of dense acetowhite lesions on the SCJ, particularly when combined with vascular changes (coarse mosaic and/or punctation). A friable lesion with an irregular surface, frequently accompanied by a dense white epithelium or atypical vessels, was defined as 'cancer'. A random biopsy was performed when cytology indicated a high risk of HSIL [i.e., when atypical squamous cells could not exclude HSIL (ASC-H), atypical glandular cell (AGC) or HSIL], even if the colposcopy sample was normal. Endocervical curettage was required when there was a type 3 transformation zone, the lesion extended to the cervical canal or when the involvement of the cervical canal could not be excluded. According to the colposcopic impressions, all HSILs verified by pathological biopsy were divided into a low-grade impressions group, which included normal, metaplasia and LSIL (n=62) and a high-grade impressions group, which included HSIL and cancer (n=74).

Statistical analysis. Data are presented as mean ± SD for continuous normal distributions; 95% confidence intervals (CI) are presented for non-normal distributions. An independent samples t-test and Mann-Whitney U test were used to compare the mean and median values between two groups. The differences in proportions between classification variables were determined by Fisher's exact test. The receiver operating characteristic curve (ROC) was used to establish the optimal critical value of colposcopy misjudgment. All two-tailed statistical tests were performed using SPSS software (version 26.0, IBM Corp.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Overview of patient data. The median age of patients was 41.5 years and there were no statistically significant differences between the low-grade and high-grade colposcopy impression groups in terms of age, cytology [≥atypical squamous cells of undetermined significance (ASCUS)], HPV 16/18 positive rate or the location of lesions (Table I). However, there were significantly fewer epithelial layers (13.7±4.8 vs. 19.1±5.5) and decreased epithelial thickness (112.0±45.1 μm vs. 153.4±49.0 μm) in the low-grade colposcopic impression group compared with the high-grade colposcopic impression group (Table I).

Association between epithelial thickness and colposcopic impressions in CIN2 and CIN3. Stratified analysis was performed to examine the association between colposcopy impression and the thickness of the epithelium in cases of CIN2 (n=79) and CIN3 (n=57) diagnosed by histology (Table II). In the CIN2 group, the mean number of epithelial layers (12.8±4.2 vs. 17.8±4.2) and epithelial thickness (105.2±41.9 μm vs. 150.3±50.0 μm) were significantly lower in the low-grade colposcopic impression group compared with the high-grade colposcopic impression group. However, there were no significant differences in either epithelial thickness or the number of epithelial layers in the CIN3 group. In the low-grade colposcopic impression groups, CIN2 lesions had significantly fewer epithelial layers (12.8±4.2 vs. 17.2±5.4) and decreased epithelial thickness (105.2±41.9 μm vs. 140.4±48.6 μm) compared with CIN3 lesions. However, in the colposcopic high-grade impressions group, there was no significant difference in the number of layers or epithelial thickness between CIN2 and CIN3. The analysis for distinguishing low-grade and high-grade colposcopy impressions by the number of epithelial layers or thickness showed that the average number of layers at the critical threshold was 16 and the epithelial thickness was 138 μm, with a sensitivity and

Table I. Clinical and pathological characteristics of high-grade squamous intraepithelial lesions with different colposcopic impressions.

Characteristic	Low-grade impressions (n=62)	High-grade impressions (n=74)	P-value
Median age, years (IQR)	38.5 (33.8-53.0)	39.5 (34.0-44.3)	0.638
High risk of cytology (≥ASCUS), n (%)	38 (61.3)	51 (68.9)	0.371
HPV16 and/or HPV18 positive, n (%)	27 (43.5)	43 (58.1)	0.121
Layers, median (mean ± SD)	13.7±4.8	19.1±5.5	<0.001
Epithelial thickness, μm (mean ± SD)	112.0±45.1	153.4±49.0	<0.001
Site of lesion, n (%)			0.884
6 o'clock involved	15 (24.2)	17 (23.0)	
12 o'clock involved	14 (22.6)	13 (17.6)	
Cervical canal involved	5 (8.1)	7 (9.5)	
Other site	28 (45.2)	37 (50.0)	

IQR, inter quartile range; ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus.

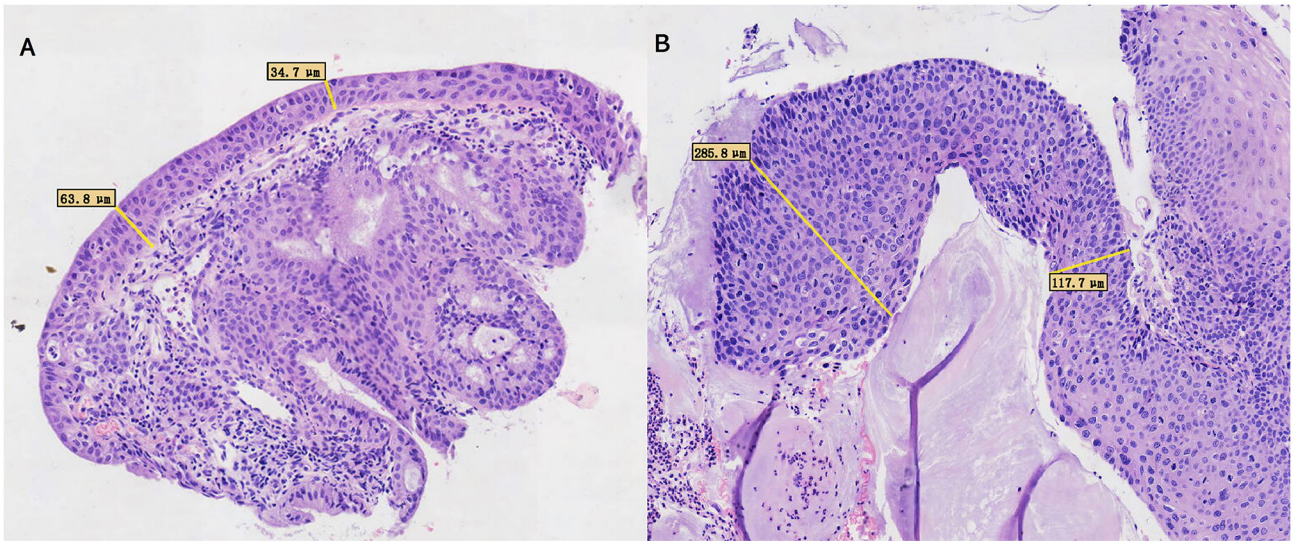

Figure 1. Representative epithelial thickness measurement. Representative example of the measurement of average epithelial thickness in (A) thin HSIL and (B) classic-type HSIL. Average epithelial thickness=[(thickness of the thickest part + thickness of the thinnest part)/2]. Tissues were previously stained with hematoxylin and eosin and observed under x200 magnification. HSIL, high-grade squamous intraepithelial lesion.

specificity of 77.4-79.0 and 60.8-68.9%, and the area under the curve was 0.774 and 0.749, respectively (Fig. 2).

Characteristics of thin HSILs. A total of 12 cases of thin HSILs were analyzed with a median patient age of 43.2±3.4 years (range, 30-62 years) (Table III). Cytological impressions of 7/12 (58.3%) thin HSILs were negative, 3/12 (25.0%) cases were low-risk (ASCUS/LSIL) and 2/12 (16.7%) cases were high-risk (ASC-H/HSIL). A total of 5 (41.7%) thin HSILs were positive for HPV 16/18 and the remaining 7 HSILs (58.3%) were positive for other HPV genotypes. Colposcopic impressions of 11 cases were low grade and below, with 7 cases of thin acetowhite changes located on the surface near the SCJ (one case is demonstrated in Fig. 3); 1 case had a lesion located outside the transformation zone; 2 cases had a normal colposcopy, with thin HSILs identified only within the endocervix; and 1 case had a nabothian cyst without a significant acetic acid white area, but upon biopsy was unexpectedly found to be CIN3. The lesion sizes of 10/12 thin HSILs under colposcopy were ≤10% of the cervical area, with isolated lesions. Examination of hematoxylin and eosin-stained sections demonstrated that the number of epithelial layers ranged from 5.5-9.0, epithelial thickness ranged from 46.2-125.1 μm and the horizontal extension was between 234-833 μm. Ten of the 12 thin HSILs were CIN2. Seven of 12 thin HSILs were located at the SCJ and 2 HSILs were located in the endocervical columnar epithelium; this distribution was in accordance with that from the colposcopy impression.

Discussion

Previous studies (5,6) are conflicting on whether false negative colposcopy is associated with epithelial thickness. This

Table II. Number and thickness of epithelial layers of high-grade squamous intraepithelial lesions with different colposcopic impressions (mean ± SD).

	Number of epithelial layers			Thickness of epithelium		
	Colposcopy impression			Colposcopy impression, μm		
Variable	≤Low grade	High grade+	P-value	≤Low grade	High grade+	P-value
CIN2 (n=79)	12.8±4.2	17.8±4.2	<0.001	105.2±41.9	150.3±50.0	<0.001
CIN3 (n=57)	17.2±5.4	19.9±6.1	0.166	140.4±48.6	155.4±48.8	0.349
P-value	0.014	0.665		0.004	0.113	

CIN, cervical intraepithelial neoplasia.

Table III. Clinical and pathological characteristics of thin high-grade squamous intraepithelial lesions.

Patient no.	Patient age at diagnosis, years	Cytology	HPV genotype	Colposcopy impression	Lesion size, % cervical area	Location of thin HSIL	Diagnosis	Number of epithelial layers	Epithelial thickness, μm	Horizontal diameter of thin HSIL, μm
1	50	NILM	HPV 52	NILM	0	Endocervix	CIN2	7.0	90.7	673
2	34	HSIL	HPV 33	LSIL	10	11˚	CIN2	7.5	74.5	833
3	36	NILM	HPV 16	LSIL	0	6˚	CIN2	5.5	125.1	459
4	62	LSIL	HPV 51,56	LSIL	5	9˚	CIN2	7.5	110. 0	529
5	41	NILM	HPV 53,31	NILM	0	Endocervix	CIN2	8.0	68.5	685
6	60	ASC-H	HPV 52	Nabothian cyst	5	Nabothian cyst	CIN3	6.5	84.2	385
7	34	NILM	HPV 42,58	LSIL	10	6˚	CIN2	9.0	53.1	234
8	59	ASCUS	HPV 16,18	LSIL	10	12˚	CIN2	7.0	75.4	327
9	41	NILM	HPV 33	HSIL	20	6˚	CIN3	7.0	71.2	692
10	40	NILM	HPV 16,68	LSIL	10	6˚	CIN2	8.0	69.5	612
11	31	NILM	HPV 16	LSIL	10	12˚	CIN2	8.0	68.3	368
12	30	LSIL	HPV 16	LSIL	20	1,5,7˚	CIN2	8.5	46.2	634

NILM, negative for intraepithelial lesion or malignancy; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; ASC-H, atypical squamous cells cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance.

controversy was addressed in the present study, by investigating the relationship between HSILs and the underdiagnosis of CIN by colposcopy. The findings of the present study suggested that the underestimation of colposcopy for high-grade lesions was associated with the number of epithelial layers and thickness. In particular, HSILs with a low-grade colposcopic impression were found to have a thinner epithelium with fewer layers compared with those with a high-grade colposcopic impression, particularly those classified as CIN2. In 12 women with thin HSILs, 91.6% received a colposcopic impression of low grade or below, which further indicated that HSIL was easily missed by colposcopy in these patients.

The presence of acetowhite changes is an important evaluation indicator used in determining a colposcopic impression (8). Low-grade lesions or metaplasia present as thin/translucent acetowhite lesions, whereas high-grade lesions demonstrate dense/thick acetowhite changes. Due to the subjectivity of colposcopy, high-grade lesions can be under- or over-diagnosed according to the acetowhite appearance. The diagnostic accuracy differed greatly when the colposcopy result was based on a CIN2+ impression or when the colposcopist speculated that there was some disease present (DP). The sensitivities were 68.5 and 95.7% and specificities were 75.9 and 34.2% for CIN2+ impression and DP, respectively (9); thus, the wide range of published diagnostic accuracy figures could be due to the use of two different methods to evaluate colposcopy findings.

The relationship between epithelial thickness and colposcopy impression was first reported by Yang *et al* (5), who found that the epithelial thickness of the cervical quadrants of patients with CIN2/CIN3 with a normal colposcopic impression (184 μm) was less than that from patients with low, high or cancer colposcopic impressions (321 μm). They concluded

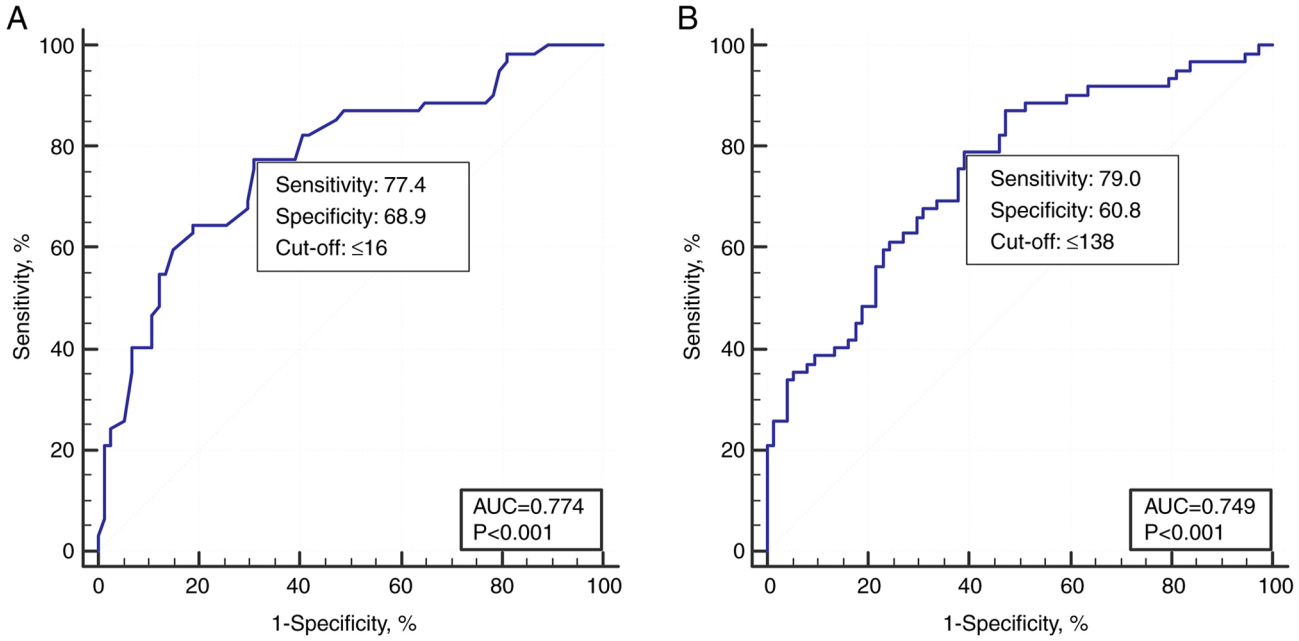

Figure 2. Receiver operating characteristic curve of optimal critical value of epithelial thickness and number of layers for colposcopy misjudgment. (A) Number of layers. (B) Thickness of epithelium. AUC, area under the curve.

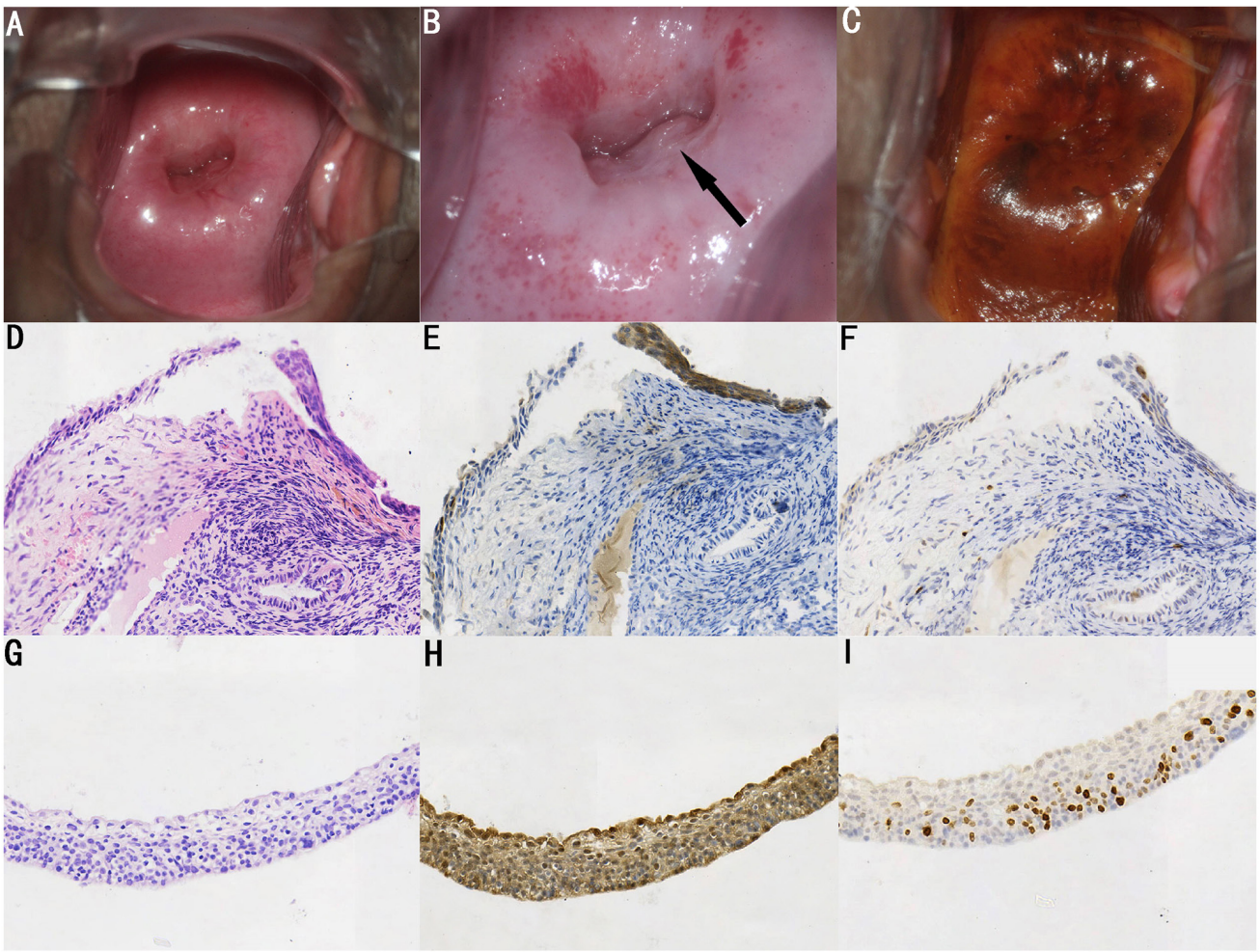

Figure 3. Colposcopy and pathological images of thin HSIL of patient 4 in Table III. Colposcopy images (A) before acetic acid, (B) after acetic acid and (C) after Lugol's iodine staining which demonstrated atrophic changes of the cervical epithelia. (D) HE-stained (x40 magnification), (E) p16 positive (x40 magnification), (F) Ki-67:15% positive (x40 magnification), (G) HE stained (x100 magnification), (H) p16 positive (x100 magnification) and (I) Ki-67 15% positive (x100 magnification). Black arrow indicates thin acetowhite change at 6 o'clock. HSIL, high-grade squamous intraepithelial lesion; HE, hematoxylin and eosin.

that colposcopists cannot see certain CIN2/CIN3 lesions associated with the thickness of the epithelium. However, Ghosh *et al* (6) reported that CIN3 lesions with high-grade abnormalities were slightly thicker than those without a visible colposcopic abnormality. Dysplasia thickness was positively correlated with the CIN grade but was reported to be unassociated with colposcopic appearance, which led to the conclusion that false-negative colposcopy with high-grade CIN was possibly caused by the failure to detect small or predominantly endocervical lesions rather than a 'thin' CIN (6).

In the present study, the epithelial thickness of HSILs with low-grade colposcopic impressions (112.0±45.1 μm) was significantly thinner than that of lesions with high-grade colposcopic impressions (153.4±49.0 μm), which supported the finding that a low-grade colposcopic impression was associated with a thin CIN. High-grade lesions were the most likely to be underdiagnosed using colposcopy when there were <16 layers and the thickness of the epithelium was <138 μm. This was similar to Yang *et al* (5) who reported that colposcopy sensitivity for CIN2/CIN3 was only 31.3% when the epithelial thickness was <139 μm. Therefore, the findings from the present study provide additional evidence for the ASSCP Evidence-Based Consensus recommendation to perform multiple biopsies targeting all acetowhitening regions, regardless of the presence of metaplasia or higher levels of abnormality (8).

Yang *et al* (5) reported that for all colposcopic impressions [normal, low-grade or high-grade (not including cervical cancer)], the epithelial thickness of lesions histologically determined to be a high grade (CIN2/3) was lower than that of those determined to be CIN1 and normal. Similar results were also reported by Wang *et al* (10). One possible explanation is that HSILs are primarily composed of enlarged nuclear atypia cells, which are smaller than cells in LSILs, so the epithelium of HSILs is thinner than that of LSILs. However, Ghosh *et al* (6) reported that the dysplasia thickness increased with CIN grade, but that there was no correlation between the total epithelium thickness and the neoplasia severity [CIN1 (271.9 μm) > CIN3 (218.5 μm) > normal histopathology (212.8 μm) > CIN2 (191.4 μm)]. Although LSILs confirmed by pathological biopsy were not included in the present study, the thicknesses of CIN2 and CIN3 lesions were compared, which indicated that the epithelia of CIN3 (152.2±48.7 μm) were thicker than those of CIN2 (121.8±49.8 μm), which was consistent with the results of Ghosh *et al* (6).

When CIN2 and CIN3 were analyzed separately, it was found that CIN2 was more likely to have false negative colposcopy impressions compared with CIN3. The number of epithelial layers and thickness of CIN2 were significantly lower for low-grade colposcopic impressions than for high-grade impressions, but there was no significant difference observed for CIN3. In addition, epithelial thickness for CIN2 (105.2±41.9 μm) was lower than that for CIN3 (140.4±48.6 μm) in the low-grade colposcopic impressions group, which indicated that CIN2 is susceptible to misdiagnosis. However, patients with CIN3 frequently presented with typical thick/dense acetowhite lesions, which were often accompanied by coarse mosaic patterns or punctation and were therefore difficult to overlook.

It has been reported that the formation of HSILs occurs via two pathways (11). Classic HSILs typically arise from LSILs after HR-HPV infection of mature stratified metaplastic squamous epithelium (MSE). HSILs can also develop from early MSE (11), these lesions (defined as thin HSILs) occur in non-stratified or very thin immature squamous epithelia, which explains why certain HSILs develop without an anteceding LSIL (11-13). The frequency of thin HSILs has been reported in only a few studies. Reich and Regauer reported that out of 25 conization specimens, 76% contained both thin HSILs and classic HSILs, 16% contained only thin HSILs and 4% contained only classic-type HSILs, and 1 (4%) contained thin HSIL and LSIL (14). The present study retrospectively analyzed 136 cases of HSILs diagnosed within a 4-month period and demonstrated that 8.8% of these cases were thin HSILs only and 41.2% were classic HSILs; among the classic HSILs, 54.8% were accompanied by thin HSILs, the majority of which occurred multifocally of the cervix. This indicates that thin HSILs combined with classical HSILs are frequent, but because the classical HSILs are more obvious, pathologists may concentrate primarily on their morphology. Thin HSILs alone are relatively rare and have a higher risk of being missed.

In previous research, 65% of HSILs were reported to be located on the SCJ, 19% were located in the endocervical columnar epithelium and 16% were found in both locations (15). Similar to the geographic distribution reported by Regauer *et al* (15), in the present study 7/12 (58.3%) of thin HSILs were located near the SCJ and 2/12 (16.7%) were located within the endocervical epithelium. Of the patients with thin HSILs, 83.3% were CIN2, 83.3% had lesions covering less than 10% of the cervical area and 91.6% had low-grade colposcopic impressions. Although thin HSILs are unequivocally diagnostic when they are between 5-9 cell layers thick, lesions with <5 cell layers often resemble immature metaplastic squamous epithelium (14,15). In the present study, the number of epithelial layers ranged from 5.5 to 9.0, with a maximum epithelium thickness of 125.1 μm, which was less than the cut-off of 138 μm, where HSIL can be easily missed by colposcopy. The challenges in colposcopic detection may be explained by the tiny dimensions of thin HSILs. Although thin HSIL may occasionally be confused with immature metaplasia with mild atypia or atypical immature metaplasia, immunohistochemically $p16^{INK4a}$ overexpression can be used as a marker to make an unequivocal diagnosis of thin HSIL (14,16).

Regarding the association with HPV, it was previously reported that 74% of thin HSILs have HR-HPV infection, among which HPV 16 was the dominant subtype, present in as many as 37% of lesions and was followed by HPV 53 (15,17). In the present study, 58.3% of thin HSILs had negative cytology and 58.3% had non-HPV 16/18 HR-HPV subtypes; these HSILs likely develop less aggressive behavior when compared with classic HSILs (15).

The strength of the present study is that it explored the confusion surrounding the relationship between underdiagnosed colposcopic impressions and epithelial thickness. The limitations of this study were that colposcopy is somewhat subjective even though the colposcopists involved had an average of 10 years colposcopy experience and had received thorough colposcopy training including colposcopy operations and terminology. In a future study, colposcopic images should be reviewed and the true causes of discrepancies evaluated through a blinded review. Finally, the accuracy of colposcopic

assessment may also be affected by additional variables, such as age, the type of transformation zone, microbiota dysbiosis and postmenopausal atrophy, which could also inevitably affect the accuracy of colposcopic assessment. These factors should also be stratified and analyzed in future studies with larger samples.

Underdiagnosis of CIN by colposcopic impressions is likely associated with thin HSIL, particularly CIN2. Thin HSILs usually present as small to minute lesions and lack the classic HSIL characteristic colposcopic impression, which may explain why this type of lesion is underdiagnosed by colposcopy. To prevent the underdiagnosis of thin HSILs, it is necessary to highlight the need for biopsy in regions with acetowhitening, even if the colposcopy impression might be metaplasia or LSIL.

Acknowledgements

Not applicable.

Funding

This work was supported by The National Key Research and Development Program of China (grant nos. 2021YFC2701200 and 2021YFC2701202) and the Research and Development Fund of Peking University People's Hospital (grant no. RDL2020-02).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ML conceptualized and designed the study and was a major contributor to writing the manuscript. XZ participated in pathological review and assessment. QZ participated in pathological section collection and pathological assessment. YZ, CZ, and JL participated in colposcopic image evaluation and review. DS organized sample collection and participated in pathological review and assessment. HT was responsible for statistical analysis and data interpretation. LW was involved in the study design, interpretation of data, revising the manuscript critically for important intellectual content, reviewing the manuscript and providing advice during the study. All authors read and approved the final version of the manuscript. ML and XZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board or Ethics Committee of Peking University People's Hospital Ethics Committee (IRB number, 2020PHB298-01; date of IRB approval, 30/10/2020).

Patient consent for publication

Written consent was obtained from the patient in respect to publishing images in Fig. 3.

Competing interests

The authors declare that they have no competing interests.

References

1. Waxman AG, Chelmow D, Darragh TM, Lawson H and Moscicki AB: Revised terminology for cervical histopathology and its implications for management of high-grade squamous intraepithelial lesions of the cervix. Obstet Gynecol 120: 1465-1471, 2012.
2. Tainio K, Athanasiou A, Tikkinen KAO, Aaltonen R, Hernándes JC, Glazer-Livson S, Jakobsson M, Joronen K, Kiviharju M, Louvanto K, *et al*: Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: Systematic review and meta-analysis. BMJ 360: k499, 2018.
3. Reid R and Scalzi P: Genital warts and cervical cancer. VII. An improved colposcopic index for differentiating benign papillomaviral infections from high-grade cervical intraepithelial neoplasia. Am J Obstet Gynecol 153: 611-618, 1985.
4. Ferris DG and Litaker MS; ALTS Group: Prediction of cervical histologic results using an abbreviated Reid Colposcopic Index during ALTS. Am J Obstet Gynecol 194: 704-710, 2006.
5. Yang B, Pretorius RG, Belinson JL, Zhang X, Burchette R and Qiao YL: False negative colposcopy is associated with thinner cervical intraepithelial neoplasia 2 and 3. Gynecol Oncol 110: 32-36, 2008.
6. Ghosh I, Mittal S, Banerjee D, Chowdhury N and Basu P: Study of correlation of cervical epithelial thickness with the grade of colposcopic abnormality. Int J Gynecol Pathol 35: 269-274, 2016.
7. Stoler M, Bergeron C, Colgan TJ, Ferenczy AS, Herrington CS and Kim KR: Squamous Cell Tumours and Precursors. Kurman RJ, Carcangiu ML, Herrington CS and Young RH (eds). WHO Classification of Tumours of Female Reproductive Organs. IARC, Lyon, pp17181, 2014.
8. Wentzensen N, Massad LS, Mayeaux EJ Jr, Khan MJ, Waxman AG, Einstein MH, Conageski C, Schiffman MH, Gold MA, Apgar BS, *et al*: Evidence-Based consensus recommendations for colposcopy practice for cervical cancer prevention in the United States. J Low Genit Tract Dis 21: 216-222, 2017.
9. Brown BH and Tidy JA: The diagnostic accuracy of colposcopy-A review of research methodology and impact on the outcomes of quality assurance. Eur J Obstet Gynecol Reprod Biol 240: 182-186, 2019.
10. Wang AC, Wang LQ, Li J, Li MX, Tu LL, Zhang YX and Liu AJ: Artificial intelligence aided measurement of cervical squamous epithelial thickness and its correlation with cervical precancerous lesions. Zhonghua Bing Li Xue Za Zhi 50: 339-343, 2021 (In Chinese).
11. Reich O and Regauer S: Two major pathways for development of high-grade squamous intraepithelial lesions of the cervix. Am J Surg Pathol 38: 1579-1580, 2014.
12. Regauer S, Reich O and Kashofer K: Cervical precancers originate from infected proliferating reserve cells: A Comparative histologic and genetic study of thin and thick high-grade squamous intraepithelial lesions. Am J Surg Pathol 46: 519-527, 2022.
13. Woodman CB, Collins SI and Young LS: The natural history of cervical HPV infection: Unresolved issues. Nat Rev Cancer 7: 11-22, 2007.
14. Reich O and Regauer S: Thin HSIL of the cervix: Detecting a variant of high-grade squamous intraepithelial lesions with a p16INK4a antibody. Int J Gynecol Pathol 36: 71-75, 2017.
15. Regauer S, Reich O and Kashofer K: Thin variant of high-grade squamous intraepithelial lesion-relationship with high-risk and possibly carcinogenic human papilloma virus subtypes and somatic cancer gene mutations. Histopathology 75: 405-412, 2019.
16. Regauer S and Reich O: CK17 and p16 expression patterns distinguish (atypical) immature squamous metaplasia from high-grade cervical intraepithelial neoplasia (CIN III). Histopathology 50: 629-635, 2007.
17. Barzon L, Militello V, Pagni S, Franchin E, Dal Bello F, Mengoli C and Palu G: Distribution of human papillomavirus types in the anogenital tract of females and males. J Med Virol 82: 1424-1430, 2010.